Gleiss & Große
Patentanwälte · Rechtsanwälte
European Patent Attorneys
European Trademark Attorneys

Intellectual Property Law
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Claims

1. Process for generating and detecting recombinant DNA sequences in prokaryotes comprising the steps of:

a) generating a first prokaryotic cell containing an extrachromosomal recipient DNA molecule, which comprises a first DNA sequence to be recombined and which can autonomously replicate in the prokaryotic cell, and an extrachromosomal donor DNA molecule, which comprises a second DNA sequence to be recombined and at least a first marker sequence encoding a gene product and which cannot autonomously replicate in the prokaryotic cell,

b) cultivating the first prokaryotic cell under selective conditions, which force the formation of a co-integrate or hybrid molecule between the recipient and donor DNA molecules and the recombination of the two DNA sequences to be recombined and which only allow the growth and/or propagation of the cell if the gene product of the first marker sequence is expressed, and

c) isolating a second prokaryotic cell grown and/or propagated under selective conditions and containing a hybrid DNA molecule with the at least first marker sequence and a first and a second recombined DNA sequence due to recombination between the first and the second DNA sequences,

wherein the prokaryotic cell is transiently or permanently deficient in the mismatch repair system.

2. Process according to claim 1, wherein the donor DNA molecule and the recipient DNA molecules are different linear or circular DNA structures, in particular different plasmids or bacteriophages.

3. Process according to any one of claims 1 or 2, wherein the donor DNA molecule does not have an origin of replication.

4. Process according to any one of claims 1 or 2, wherein the donor DNA molecule has a non-functional origin of replication.

5. Process according to any of claims 1 to 4, wherein the donor DNA molecule is a *Bacillus subtilis* plasmid, which cannot replicate in *E. coli*.

6. Process according to claim 5, wherein the donor DNA molecule is the *B. subtilis* plasmid pMIX91 comprising the $spec^R$ marker and the $phleo^R$ marker or the *B. subtilis* plasmid pMIX101 comprising the tc^R marker.

7. Process according to any one of claims 1 to 6, wherein the first marker sequence of the donor DNA structure is selected from the group consisting of a nutritional marker, an antibiotic resistance marker and a sequence encoding a subunit of an enzyme.

8. Process according to claim 7, wherein the gene product of the first marker sequence confers resistance to an antibiotic to a cell which is sensitive to that antibiotic.

9. Process according to claim 7 or 8, wherein the first marker sequence is $spec^R$, the gene product of which confers to a cell resistance to spectinomycin, or $phleo^R$, the gene product of which confers

to a cell resistance to phleomycin, or tc^R, the gene product of which confers to a cell resistance to tetracycline.

10. Process for generating and detecting recombinant DNA sequences in prokaryotes comprising the steps of:

d) generating a first prokaryotic cell containing an extrachromosomal recipient DNA molecule, which comprises a first DNA sequence to be recombined and which can autonomously replicate in the prokaryotic cell, and an extrachromosomal donor DNA molecule, which comprises a second DNA sequence to be recombined and at least a first marker sequence encoding a gene product and which cannot autonomously replicate in the prokaryotic cell,

e) cultivating the first prokaryotic cell under selective conditions, which force the formation of a co-integrate or hybrid molecule between the recipient and donor DNA molecules and the recombination of the two DNA sequences to be recombined and which only allow the growth and/or propagation of the cell if the gene product of the first marker sequence is expressed, and

f) isolating a second prokaryotic cell grown and/or propagated under selective conditions and containing a hybrid DNA molecule with the at least first marker sequence and a first and a second recombined DNA sequence due to recombination between the first and the second DNA sequences,

wherein the donor DNA molecule is the *B. subtilis* plasmid pMIX91 comprising the *spec*^{R} marker and the *phleo*^{R} marker or the *B. subtilis* plasmid pMIX101 comprising the *tc*^{R} marker.

11. Process according to claim 10, wherein the recipient DNA molecule is a linear or circular DNA structure, in particular a plasmid or a bacteriophage.

12. Process according to claim 10 or 11, wherein the prokaryotic cell has a functional mismatch repair system.

13. Process according to claim 10 or 11, wherein the prokaryotic cell is transiently or permanently deficient in the mismatch repair system.

14. Process according to any one of claims 1 to 13, wherein the recipient DNA molecule is a plasmid, which can replicate in *Escherichia coli*.

15. Process according to claim 14, wherein the recipient DNA molecule is the *E. coli* plasmid pACYC184 or the *E. coli* plasmid pMIX100 or a derivative thereof.

16. Process according to any one of claims 1 to 15, wherein the donor DNA molecule and/or its origin of replication are derived from a prokaryotic species other than the prokaryotic species in cells of which the donor DNA molecule is introduced.

17. Process according to any one of claims 1 to 16, wherein the function of the origin of replication of the donor DNA is impaired by a mutation.

18. Process according to any one of claims 1 to 17, wherein the donor DNA molecule contains a second marker sequence.

19. Process according to any one of claims 1 to 18, wherein the recipient DNA molecule contains a third marker sequence and optionally a fourth marker sequence.

20. Process according to claim 18 or 19, wherein the second, third and fourth marker sequences are protein-coding or non-coding sequences selected from the group consisting of nutritional markers, pigment markers, antibiotic resistance markers, antibiotic sensitivity markers, restriction enzymes sites, primer recognition sites and sequences encoding a subunit of an enzyme.

21. Process according to claim 20, wherein the gene products of the third and fourth marker sequences of the recipient DNA molecule confer resistance to an antibiotic to a cell which is sensitive to that antibiotic.

22. Process according to claim 21, wherein the gene product of the third marker sequence confers to a cell resistance to tetracycline.

23. Process according to claim 21, wherein the gene product of the fourth marker sequence confers to a cell resistance to chloramphenicol.

24. Process according to any one of claims 1 to 23, wherein the first and the second DNA sequences to be recombined diverge by at least two nucleotides.

25. Process according to any one of claims 1 to 24, wherein the first and the second DNA sequences to be recombined are naturally occurring sequences.

26. Process according to claim 25, wherein the first and/or the second DNA sequences to be recombined are derived from viruses, bacteria, plants, animals and/or human beings.

27. Process according to any one of claims 1 to 24, wherein the first and/or the second DNA sequences to be recombined are artificial sequences.

28. Process according to any one of claims 1 to 27, wherein each of the first and the second DNA sequences to be recombined comprises one or more protein-coding sequences and/or one or more non-coding sequences.

29. Process according to any one of claims 1 to 28, wherein the first prokaryotic cell is generated by simultaneously or sequentially introducing the recipient DNA molecule and the donor DNA molecule into a prokaryotic cell.

30. Process according to claim 29, wherein the recipient and donor DNA molecules are introduced into the prokaryotic cell via transformation, conjugation, transduction, sexduction and/or electroporation.

31. Process according to any one of claims 1 to 30, wherein the first prokaryotic cell is cultivated in the presence of at least one antibiotic to which the gene product of the first marker sequence confers resistance.

32. Process according to claim 31, wherein the first prokaryotic cell is additionally cultivated in the presence of a second, a third and/or a fourth antibiotic to which the gene products of the second marker sequence, the third marker and the fourth marker sequence, respectively, confer resistance.

33. Process according to any one of claims 1 to 32, wherein the prokaryotic cell is a cell of an archaebacterium or an eubacterium.

34. Process according to claim 33, wherein the eubacterium is a gram-negative bacterium, a gram-positive bacterium or a cyanobacterium.

35. Process according to claim 34, wherein the gram-negative bacterium is *Escherichia coli*.

36. Process according to claims 1 to 9 and 13, wherein the transient or permanent deficiency of the mismatch repair system is due to a mutation, a deletion, and/or an inducible expression or repression of one or more genes involved in the mismatch repair system, a treatment with an agent that saturates the mismatch repair system and/or a treatment with an agent that globally knocks out the mismatch repair.

37. Process according to claims 1 to 9, 13 and 36, wherein the prokaryotic cell has a mutated *mutS* gene and/or mutated *mutL* gene.

38. Process according to any one of claims 1 to 37, wherein the first and the second recombined DNA sequences contained in the hybrid DNA molecule of the second prokaryotic cell are selected and/or isolated and/or analysed.

39. Process according to claim 38, wherein the first and the second recombined DNA sequences are isolated by restriction enzyme cleavage.

40. Process according to claim 38, wherein the first and the second recombined DNA sequences are amplified by PCR.

41. Process according to any one of claims 38 to 40, wherein the isolated first and second recombined DNA sequences are inserted into a donor DNA molecule and a recipient DNA molecule, respectively, and subjected to another round of recombination.

42. *Bacillus subtilis* plasmid pMIX91 which comprises the $spec^R$ marker and the $phleo^R$ marker and the restriction sites *Sca*I, *Ppu*MI and *Eco*O109I for inserting a foreign DNA sequence.

43. *Bacillus subtilis* plasmid pMIX101 which comprises the tc^R marker sequence and the restriction sites *Xho*I and *Pst*I for inserting a foreign DNA sequence.

44. Use of the *B. subtilis* plasmids pMIX91 or pMIX101 as donor DNA molecules in a process according to any one of claims 1 to 41 for generating and/or detecting recombinant DNA sequences in a prokaryotic host cell, preferably in an *E. coli* cell.

45. Use of the *E. coli* plasmids pACYC184 or pMIX100 or a derivative thereof as recipient DNA molecule in a process according to any one of claims 1 to 41 for generating and/or detecting recombinant DNA sequences in a prokaryotic host cell, preferably in an *E. coli* cell.

46. Kit comprising at least a first container which comprises cells of the *E. coli* strain AB1157 or the *E. coli* strain MXP1 or the *E. coli* strain DHB10, a second container which comprises cells of the *E. coli* strain AB1157 containing plasmid pACYC184 or cells of the *E. coli* strain DHB10 containing plasmid pMIX100 and a third container comprising cells of the *B. subtilis* strain DSM4393 containing plasmid pMIX91 or cells of the *B. subtilis* strain 1A423 containing plasmid pMIX101.

47. Kit comprising at least a first container which comprises cells of the *E. coli* strain AB1157 or the *E. coli* strain MXP1 or the *E. coli* strain DHB10, a second container comprising DNA of plasmid pACYC184 or plasmid pMIX100 and a third container comprising DNA of plasmid pMIX91 or plasmid pMIX101.

48. A process for producing a hybrid gene and/or a protein encoded by a hybrid gene in a prokaryotic cell, wherein a process according to any one of claims 1 to 41 is carried out and the hybrid gene and/or the protein encoded by the hybrid gene is produced in the prokaryotic cell and the hybrid gene and/or the encoded protein is selected in the prokaryotic cell and/or isolated therefrom after expression.